EXHIBIT

Motor impairments represent the most common form of disability resulting from stroke. Depending on the severity of the impairments, motor rehabilitation can result in significant improvements in motor function over time. The efficacy of the phosphodiesterase inhibitor HT0712 ((3S, 5S)-5-(3-cyclopentyloxy-4-methoxy-phenyl)-3-(3-methyl-benzyl)-piperidin-2-one; also known as IPL 455,903)) in promoting rehabilitation-dependent motor recovery and enhancing functional restoration within the motor cortex following cortical ischemia was examined.

Materials and Methods

Subjects: Forty-two adult (90 days) male Long-Evans hooded rats (350-420g) were group housed (2 animals/cage) in standard laboratory cages on a 12:12 hour light dark cycle throughout the experiment.

Reach Training: Over the course of several days, all animals were placed on a restricted diet until they reached 90% of their original body weight. A brief period of pretraining was then given to familiarize the rats with the reaching task. This involved placing the animals into test cages (10 X 18 X 10 cm) with floors constructed of 2 mm bars, nine mm apart edge to edge. A four cm wide and 5 cm deep tray filled with food pellets (45 mg; Bioserv) was mounted on the front of the cage. The rats were required to reach outside the cage and retrieve pellets from the tray. All rats remained in pretraining until they had successfully retrieved 10 pellets (approximately 1 hour/day for 2 days). After pretraining, the rats were placed into a Plexiglas cage (11 cm X 40 cm X 40 cm) with a 1 cm slot located at the front of the cage. Animals were trained for 20 minutes each day to reach through the slot and retrieve food pellets from a table outside the cage. Rats were permitted to use either limb and the preferred limb was noted for each animal. Each session was videotaped and later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches [(# successful retrievals/the total # of reaches) x 100] was then calculated. Animals were trained for

approximately 2 weeks on this task to establish a baseline measure of motor performance. Baseline was defined as the average accuracy across the three final days of training. Post stroke performance was expressed as a percentage of the baseline performance.

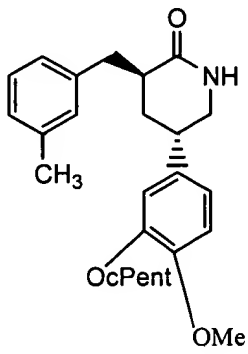
Electrophysiological Mapping: Within 2 days of the final training session, standard intracortical microstimulation (ICMS) techniques were used to generate detailed maps of forelimb regions of the motor cortex contralateral to the trained forelimb. Prior to surgery animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.). Animals received low levels of isofluorane (0.15%) and supplemental doses of ketamine (20 mg/kg i.p.) as needed. Under sterile conditions, a craniotomy was performed over the motor cortex contralateral to the trained paw of each animal. To prevent edema, a small puncture was made in the cisterna magna prior to removing the skull and dura. The exposed cortex was then covered in warm saline (37°C). A digital image of the cortical surface was taken and a 375 □m grid was superimposed onto the image. A glass microelectrode (controlled by a hydraulic microdrive) was used to make systematic penetrations across the cortex using the cortical surface image and grid as a guide. At each penetration site, the electrode was lowered to approximately 1550 μm (corresponding to cortical layer V). Stimulation consisted of 13, 200 μs cathodal pulses delivered at 350 Hz from an electrically isolated stimulation circuit. Animals were maintained in a prone position with the limb consistently supported. Sites where no movement was detected at ≤ 60 μA were recorded as unresponsive. Forelimb movements were classified as either distal (wrist/digit) or proximal (elbow/shoulder) and representational maps were generated from the pattern of electrode penetrations. The caudal forelimb area (CFA) was defined by a medial boundary of vibrissa representations, a lateral and caudal boundary of non response sites and a rostral boundary of head and neck representations. An image analysis program (CANVAS v. 3.5) was used to calculate the area extent of the caudal forelimb area (CFA).

Focal Infarction: Focal ischemic infarcts were created within caudal forelimb area via bipolar electrocoagulation of the surface vasculature. The infarct targeted primarily the distal forelimb representations but in some cases included small regions of proximal representations. The coagulated vessels included fine arterial and venous capillaries as well as larger vessels but specifically avoided any bypassing arteries supplying other cortical areas. Coagulation was

continued until all vessels within the targeted area were no longer visible and the tissue appeared white.

Motor Rehabilitation: Within three days of the initial mapping and infarction procedure, all animals were placed into a motor rehabilitation program that consisted of being trained daily for 15 minutes on the skilled reaching task described above for 10 days. Animals were also randomly assigned to one of five doses of HT0712: Vehicle (n=8), 0.10 mg/kg (n=7), 0.15mg/kg (n=8), 0.30 mg/kg (n=9), and 0.10 mg/kg given twice per day. All animals received injections 20 minutes prior to the daily training session with the exception of one group of 0.10 mg/kg animals that received a second injection 3 hours after training. The sessions were video taped and reaching accuracy was assessed as described above.

HT0712 has the following formula:



wherein "Me" means "methyl" and "cPent" means "cyclopentyl". HT0712 can be prepared using the methodology provided in U.S. Patent No. 6,458,829B1.

Assessing Cortical Dysfunction: Within one day of the final training session, ICMS was again used to generate a second map of the caudal forelimb area (CFA) contralateral to the trained forelimb. Prior to surgery animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.), receiving xylazine (0.02 mg/kg i.m.) and ketamine (20 mg/kg i.p.) as needed. Further, animals were placed on isoflurane (.15%, 1.5% O_2) when needed. The dental polymer, gel film and gel foam were removed and the exposed cortex covered in warm silicon oil. Mapping procedures were identical to those used in the initial mapping.

Results

Skilled Reaching: A repeated measures Analysis of variance (ANOVA) with DAY as a within subjects factor and CONDITION as a between subject factor revealed a significant DAY X CONDITION interaction [$F(9,36) = 1.72$; $p<0.05$) on reaching performance. Subsequent multiple comparisons (Fishers PLSD; $p <0.05$) showed the 0.15 mg/kg and 0.10 twice per day HT0712 injected animals to have a significantly higher reaching accuracy than all other groups during the later stages of training. The 0.10 mg/kg animals had significantly better reaching accuracies than the Vehicle and 0.30 mg/kg HT0712 animals.

Map Area: An analysis of variance with CONDITION as a between subject factor revealed a no significant effect of CONDITION on Map 1 area [$F (4,36) = 1.1$ $p>0.05$]. A significant main effect of CONDITION on Map 2 [$F(4,36) = 6.5$; $p<0.05$]. Subsequent multiple comparisons (*Fishers PLSD; $p<0.05$) showed the 0.10 mg/kg had significantly 0.10 mg/kg twice/day and the 0.15 mg/kg all had significantly larger Map 2 than the 0.30 mg/kg and Vehicle injected animals.

Discussion

Functional impairments following brain injury are due to both the loss of tissue within the damaged area and concomitant dysfunction within other brain area. The results of the present study show that recovery, which is associated with a reinstatement and reorganization of function within residual tissue, can be upregulated via the inhibition of phosphodiesterase in combination with rehabilitative training. Further, the expansion of movement representations within peri-infarct areas was accompanied by enhanced motor recovery. The increase in peri-infarct motor map area represents the restoration of cortical circuitry that is augmented through the upregulation of cAMP. The results of the present study demonstrate a dose dependent increase in motor recovery and enhanced functional restoration within motor cortex. With the exception of the animals receiving 0.30 mg/kg, all animals receiving HT0712 in combination with motor

rehabilitation had significantly larger motor maps and better post-stroke reaching performance than vehicle injected controls.

The results indicate that HT0712 contributes to recovery of motor function by augmenting the restoration of cortical function that occurs during rehabilitation. Specifically, HT0712 may act to facilitate synaptic strengthening and the reinstatement of the cortical circuitry required to support both the motor maps and skilled motor behavior.